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Effects of particle size on starling preference for food coated with activated charcoal

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Using European starlings (*Sturnus vulgaris*) as a model, the intake of feed blended with different sized particles of activated charcoal was monitored in one- and two-cup feeding trials. Charcoal particles of the same size class as the food (1.7–0.25 mm) did not affect consumption by birds. Birds decreased consumption of food coated with charcoal particles of 0.25–0.106 mm. Coating the food with ultra-fine particles, <0.106 mm, did not affect food intake. The hypothesis that activated charcoal exerts its repellent effect due to organic absorbency characteristics is rejected, because if true, all size classes tested should have suppressed food consumption. Coating food with particles of intermediate size may change the textural characteristics of the food such that it is recognized as grit, not as food. Ultra-fine coatings of food are perceptually perceived as smooth. Thus, reduced consumption of treated food does not reflect repellency *per se*. Rather, reduced intake reflects a change in perception of the experimental food particle and consumption rate is commensurate with intake of non-nutritive particles, i.e. grit. Contingent upon further evaluation, this trait might be exploited for the non-chemical protection of seeds and granular pesticides.

Keywords: activated charcoal, grit, particle size, repellent, starling, *Sturnus vulgaris*

Few non-lethal repellents are registered for the control of avian depredation (Mason and Clark, 1992). However, a few studies have focused attention on the possible use of particulate coatings as seed treatments. Decker and Avery (1990) reported marginal success in protecting rice seeds from blackbird depredation using clays. Dolbeer and Ickes (1994) found similar protection offered by coating rice seed with plaster and portland cement. Best and Gionfriddo (1994a) found that house sparrows (*Passer domesticus*) avoided gypsum-based pesticide particles coated with graphite. Mason and Clark (1994, 1995) found that feed and turf treated with activated charcoal suppressed consumption of treated material by starlings (*Sturnus vulgaris*) and snow geese (*Chen caerulescens*), respectively.

During the course of more detailed investigations into effective carriers for chemical repellents, I observed patterns of protection offered by charcoal particle coatings that did not emerge in previous studies of particulates. These experiments present data that examine the possibility that particle size of the charcoal coating influences diet choice.

Materials and methods

Birds

Starlings were decoy-trapped in Sandusky, Ohio and transported to the Monell Center. Upon arrival, the birds were group housed and adapted to laboratory conditions for at least a 2-week period, i.e., temperature 23°C, light:dark cycle 12:12 h. Birds were given free access to non-medicated Bird Conditioner (referred to below as 'food'; Burlington Mills, Bucks County, PA, USA) and tap water.

Test substance

Activated charcoal (CAS No. 64365–11–3) was selected as the test substance because previous studies showed that it was an effective particulate repellent (Mason and Clark, 1994). Standard 12–60 mesh charcoal was used as stock material. Smaller particles were achieved by grinding the stock material with a mortar and pestle. Particles were sequentially sorted into discrete mesh categories with standard wire sieves. In all, five particle size ranges were prepared, corresponding to mesh sizes of: 14–60 (1.7–0.25 mm), 60–100 (0.25–0.15 mm), 100-140 (0.15–0.106 mm), 140–230 (0.106–0.063 mm), and 230+ (<0.063 mm). Charcoal was blended with food to achieve a 5% mixture (g/g). A sixth treatment category, untreated food, served as the control. Food particles fell within the range of 12–60 mesh.

Activated charcoal is electrostatically charged. This property allows charcoal to cling to the surface of substrates, i.e. food. However, the mass of larger particles (>0.25 mm) is sufficient to allow gravitational forces to over come the electrostatic attraction of charcoal to food. Thus, such particles settle out. As a result, food and charcoal particles are intermingled after blending. Smaller particles (<0.25 mm) do not have sufficient mass, thus electrostatic forces are stronger than gravitational forces resulting in food particles becoming coated with charcoal after blending.

Experiment 1: One-cup feeding trials

Twenty-four starlings were drawn randomly from group housing and adapted to experimental conditions for one week. Starlings were housed individually (61 × 36 × 41 cm) and visually isolated from conspecifics. At 0900 h on each of four pre-treatment days, all birds were presented with a single cup (8 cm diameter), each containing 20 g of plain food. Each cup had a metal lid with a 3.8 cm hole in the center through which the birds could feed. The lid kept faeces from contaminating food and minimized spillage. Birds had free access to water during the course of the test. After 2 h, the remaining food in each cup was weighed (±0.1 g). The 2 h test was used so that the data would be comparable to data collected for other repellent substances (e.g. Mason *et al.*, 1984). At the end of the trial birds were allowed free access to food and water. Prior to lights-out, food was removed from the cages, and birds were food-deprived overnight to ensure measurable consumption during test periods. The food deprivation regime remained in effect throughout the experiment.

After the fourth pre-treatment session, birds were assigned randomly to one of six groups (n = four/group). As a prerequisite to initiating the experimental trials, similarity of consumption among groups was verified with a two-way analysis of variance with group as the between measures effect and day as a repeated measure. Having met the criterion condition, the test was initiated with the protocol described above.

Experiment 2: Two-cup feeding trials

Twenty-four starlings were drawn randomly from group housing and adapted to experimental conditions as outlined in Experiment 1. The only difference in the protocols occurred for presentation of the food-cups. During each 2 h trial, birds were presented with two food cups bound together with a rubber band. During the pre-treatment period 20 g of food was available in each food cup. During the treatment period 20 g of plain food and 20 g of charcoal-treated food were available. The position (right/left) of the adulterated food was determined by a counter-balanced design (i.e. alternated across birds and days), such that any strong position effects would result in a score of indifference for food consumption. Consumption is reported as a preference score: g treated food consumed divided by the sum of all food (g treated and g untreated) consumed. A score of 1.0 indicated total preference for charcoal adulterated food, a score of 0.5 indicated indifference to the treatment effect, and a score of zero indicated a complete avoidance of charcoal adulterated food.

Analysis

The criterion condition of similarity of group consumption during the pretreatment period for the one-cup test (total food intake) and the two-cup test (preference score) was evaluated separately with a two-factor analysis of variance (ANOVA). The between measures factor in these analyses was group (six levels), while day (four levels) was the repeated (within subjects) measure. Evaluation of particle size effects during the treatment phase of the experiments was carried out with the same statistical method. A least significance difference *post hoc* test was used to test for differences among group means for the one-cup and two-cup feeding experiments. A modified Dunnet's test (Winer, 1971) was used for the two-choice experiment to test the hypothesis that individual group preference did not differ from a score of 0.5, i.e. indifference (Clark and Shah, 1991).

Results

Experiment 1: One-cup test

Food consumption among groups was similar during the pretreatment period ($p > 0.05$). Because the criterion condition was met, the experiment testing for charcoal effects on food consumption proceeded. The pattern of food consumption across days was similar for all groups ($F = 1.63$, df = 15, 54, $p = 0.095$). Neither was there a group/particle size effect (*Figure 1*; $F = 2.32$, df = 5, 18, $p = 0.086$). However, there was a numerical tendency for consumption to be lower for birds presented with food treated with charcoal particle sizes of 0.25–0.15 mm (*Figure 1*). The larger variance associated with this treatment level was due to the almost complete avoidance of treated food for two birds, while the remaining two birds maintained normal appetite for the food. Food consumption varied as a function of time ($F = 2.82$, df = 3, 54, $p = 0.048$). The *post hoc* test showed that food intake differed between the first (the day of lowest intake) and the fourth day (the day of highest food intake). There were no other differences for food consumption among days.

Experiment 2: Two-cup test

Food consumption among groups in the two-cup test was similar during the pretreatment period ($p > 0.05$). Having met the criterion condition, the experiment testing for charcoal effects on food consumption pro-

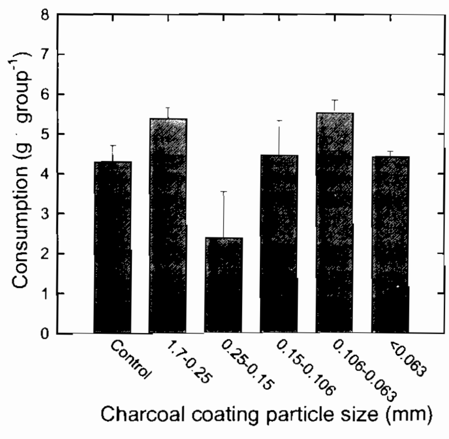

Figure 1. The consumption of food (g/group, n = 4) as a function of particle size of the charcoal coating in a one-cup test with starlings. The vertical bars depict one standard error

ceeded. There was no interaction effect between day and treatment ($F = 1.29$, df = 15, 34, $p = 0.243$), nor was there a significant main effect for day ($F = 0.99$, df = 3, 54, $p = 0.403$). However, there was a significant group/particle size effect ($F = 3.00$, df = 5, 18, $p = 0.039$). Starlings avoided food treated with intermediate sized particles of activated charcoal (*Figure 2*). The Dunnet's test showed that only those groups presented with food adulterated with charcoal particle sizes of 0.25–0.15 and 0.15–0.106 mm had preference scores different from a score of 0.5 ($p < 0.05$). The least squares *post hoc* test for the ANOVA showed that consumption was lower for food treated with a charcoal particle sizes 0.25–0.15 mm relative to consumption for the 1.7–0.25 mm, <0.063 and control groups (*Figure 2*, inset). Consumption of food treated with a charcoal particle size of 0.15–0.106 mm was lower relative to consumption for the 1.7–0.25 mm, and <0.063 mm treatment groups. There were no other group differences for consumption.

Discussion

Inclusion of grit particles in the avian diet, whether incidental (Reeder, 1951) or by active selection (Kilham, 1960), is well documented (Best and Gionfriddo, 1991). Because grit can have nutritional significance, its selection may be based upon composition. For example, calciferous grit may be preferred to supplement dietary calcium requirements necessitated by egg shell synthesis (Sadler, 1961; Kopischke, 1966). More generally, dietary grit is presumed to facilitate disintegration of food in the gizzard (May and Braun, 1973). However, at least some birds maintained on a grit-free diet are not adversely affected (Beer and Tidyman, 1942). This

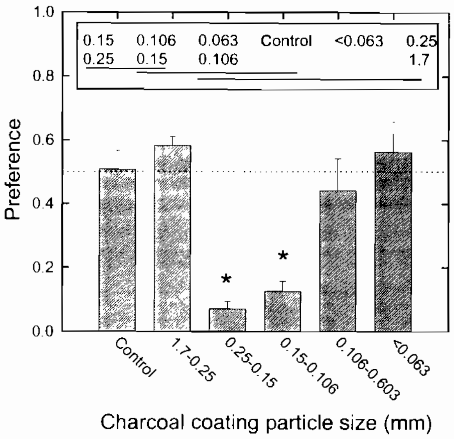

Figure 2. The preference scores as a function of particle size of the charcoal coating in a two-cup test with starlings. A score of 1.0 indicates absolute preference for charcoal-coated food relative to the cup containing plain food, a score of zero indicates a complete avoidance of charcoal-treated food relative to the cup containing plain food, and a score of 0.5 (dashed line) indicates indifference between the charcoal treatment and the cup containing plain food. Vertical bars depict one standard error. Asterisks depict statistical difference ($p < 0.05$) between the preference value of the treatment level and the indifference score of 0.5. Inset. The rank order of mean preference scores for the treatment levels (smallest to highest). Lines connect treatment levels of similar means

latter finding should not be surprising in view of the well developed musculature of the gizzard in many species of birds (Schorger, 1960).

Dietary preferences based upon particle sizes are known, and are presumed to be the result of selection for particle sizes that increase bill handling and manipulation efficiency (Kooloos, 1986). Moreover, the optimum particle size would be expected to allometrically scale across species as a function of bill size and morphology (Hespenheide, 1973). It appears that such species-specific selection also occurs for selection of grit particle sizes (Best, 1992), and that the preference is log-linearly related to avian body mass (Best and Gionfriddo, 1991). To what extent the allometric relationships between food and grit particle sizes co-vary is unknown. Neither is it known how any putative covariance is affected by foraging mode, e.g. granivory vs omnivory. However, information on optimal diet sizes for particulate food items, e.g. seeds, might be useful indicators of preferences for grit particle size. For example, the median selected particle size of cracked corn for red-winged blackbirds (*Agelaius phoencieus*) is 1.7 mm (Mason *et al.*, 1984), while the median selected particle size of grit is 1.6 mm (Best and Gionfriddo, 1991).

Notwithstanding reasons underlying size-based selection of isolated grit particles, it is curious that optimally-sized food coated with certain sized grit particles should be avoided. Clearly the particle coating must alter the bird's perception of the food, rendering it unpalatable. Mason and Clark (1994) argued that repellency for food treated with activated charcoal particles was principally due to the organic absorbance capacity of the particles. Organic absorbency is the capacity to bind organic molecules to the surface of the substrate particle, and this is the principal reason activated charcoal is used as an organic filter in a variety of applications, e.g. gas masks. Mason and Clark (1994) found that other particle coatings with more limited organic absorbance capacity also showed some repellent effect. Coatings with no organic absorbance capacity had no effect on food consumption. Although an attempt to control for particle size was made, there were differences among the treatments. No attempt was made to control for particle shape.

Based upon the present findings it is clear that factors other than organic absorbency must affect palatability. The organic absorbance capacity of activated charcoal is a function of it electrostatic properties and it surface area. Because smaller particles have more surface area per unit volume, and more particles can attach to the surface per unit area one might expect the repellent effect to increase as particle size decreases. There was no such montonic trend in these experiments. The lack of a repellent effect for activated charcoal particles nearly equal in size to the food itself (1.7–0.25 mm) is not surprising. The electrostatic forces are not sufficiently strong to bind such particles to the surface of the food relative to their mass. Thus, such particles will intermingle with the food but not coat it. One might plausibly argue that these charcoal particles do not diminish the palatability of food because the birds are able to actively avoid the charcoal without influencing food consumption. One might also plausibly argue that birds eat this sized charcoal particle in proportion to its occurrence in the food. The latter argument would contradict the organic absorbency hypothesis, while the former would support the hypothesis. Lack of effect for this treatment level neither argues for or against the organic absorbency hypothesis because of the uncertainty one has about the nature of the bird's contact with the charcoal particle and whether there exists preferential selection of particles based upon type. The consumption pattern for intermediate sized particles is consistent with the organic absorbency hypothesis. Particles of less than 0.25 mm in diameter have sufficient electrostatic charge to cling to surfaces. Thus, when such particles are blended or mixed with food, the surface of the food is readily coated. When applied as a food coating, intermediate particle sizes (0.250–0.106 mm) of activated charcoal function as repellents. Surprisingly, if the food is coated with ultra small particles of activated charcoal (<0.106 mm) palatability is not diminished, even though, relative to the 0.25–0.106 mm treatments, birds have the same chance of encountering a charcoal-coated food particle. This observation is counter to the expectation predicted by the organic absorbency hypothesis. Taken together these results suggest factors other than organic absorbance capacity are important for rendering food adulterated with specific particle sizes of charcoal unpalatable.

Avoidance of food treated with different sized particles may be attributed to sensory textural evaluation of the food to be consumed. Textural influences on palatability of food particles involves a complex sensory evaluation involving estimates of elasticity and frictional characteristics. Upon and down motion of irregular shaped objects impressed upon the sensory field, as well as irregular vibratory patterns across the sensory field, contribute to the perception of 'grittiness' (Katz, 1925; Gibson, 1967; Ringel, 1970). These properties can be quantitatively correlated to preference (Kapsalis *et al.*, 1973). From a hedonic perspective, combinations of specific values of these attributes can be classified as possessing certain 'mouth-feel'. For example, in humans smoothness is perceived as the absence of granularity, grittiness or lumpiness (Birch, Brennan and Parker, 1977), where very smooth textures are associated with viscosities in excess of 50 centiPoise. Birds, with their highly innervated bills (Zeigler, Karten and Green, 1969; Dubbeldam, Brauch and Don, 1981), are also quite capable of making such discriminations about food (Zeigler, 1976; Zweers, 1979; Kooloos, 1986). During the static phase of feeding, a particle is evaluated for hardness, size, position, shape, weight, texture and taste (Zweers, 1985). Texture may convey information about the nature of the particle. For example, Best and Gionfriddo (1994b) found that house sparrows and northern bobwhite preferred irregularly shaped over smooth particles as grit. Particles with a 'gritty' texture may be perceived as undigestible non-food items, but are acceptable on a limited basis to assist in the digestion of food particles. Particles with other elastic properties may be perceived as food items. In the present studies, ultra-small charcoal particles coating food may confer a silky or smooth texture to the coated item. In contrast, intermediate sized charcoal particles coating food may confer a gritty or abrasive texture to the coated item. Thus, intermediate sized grit coatings may camouflage food items in effect by changing the bird's sensory perception of the characteristics and nature of the item.

Several questions remain unresolved and warrant further investigation. First, the avoidance of specific size classes of grit may extend to other materials as well, e.g. quartz, silica, calcium sulfate. For example, the marginal repellent effect of quartz sand (Mason and Clark, 1994) might be amplified if specific size classes of particles are tested. Second, if avoidance for other materials is also found to vary as a function of particle size, then it would be of interest to determine how the physical characteristics of a particle coating, e.g. elasticity, hardness, affects palatability. Third, it may be of interest to determine whether there is a functional basis for rejection of certain particle sizes. For example, the interaction of particle size and physical characteristics may influence intestinal scarring, gut motility rates, absorption efficiency or gut pH. Feed particle size can effect pH and development of gastrointestinal organs in broilers (Nir *et al.*, 1994). Finally, it will be of interest to determine how particle size, texture and other physical properties affect palatability as a function of target species. From a management perspective, this latter consideration is of critical importance if particulate coatings are to have any role in strategies designed to protect seeds from bird depredations.

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